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(54) **Title:** ANTIBODIES FOR INHIBITING BLOOD COAGULATION AND METHODS OF USE THEREOF

H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTCACCATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C

CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L

CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T

AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAAATTATTACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y

AGTTCTCCATTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

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(57) **Abstract:** Disclosed is a method for treating blood coagulation in a mammal that has or is suspected of having septic shock syndrome. In one embodiment, the method includes administering to the mammal an effective amount of an antibody that binds tissue factor in a way that excludes Factor X (FX) binding. The invention has a wide range of useful applications including use to inhibit unwanted blood coagulation associated with sepsis.

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ANTIBODIES FOR INHIBITING BLOOD COAGULATION AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of USSN 10/293,417 as filed on November 12, 2002, which application is a continuation of USSN 09/293,854 as filed on April 16, 1999 (now U.S. Pat. No. 6,555,319), which application is a continuation of USSN 08/814,806 (now U.S. Pat. No. 5,986,065) as filed on March 10, 1997. The disclosures of the USSN 10/293,417 and U.S. Pat. Nos. 6,555,319 and 5,986,065 are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to novel antibodies and methods of using the antibodies to inhibit blood coagulation. In particular, the invention relates to novel antibodies that can specifically bind native human tissue factor with high affinity. The antibodies of the invention are useful for a variety of applications, particularly for reducing blood coagulation *in vivo*.

2. Background

Blood clotting assists homeostasis by minimizing blood loss. Generally, blood clotting requires vessel damage, platelet aggregation, coagulation factors and inhibition of fibrinolysis. The coagulation factors act through a cascade that relates the vessel damage to formation of a blood clot (see generally L. Stryer, *Biochemistry*, 3rd Ed, W.H. Freeman Co., New York; and A.G. Gilman et al., *The Pharmacological Basis of Therapeutics*, 8th Edition, McGraw Hill Inc., New York, pp. 1311-1331).

There is general agreement that factor X (FX) activation to factor Xa (FXa) is a critical step in the blood coagulation process. Generally, FX is converted to FXa by forming a catalytically active complex that includes "tissue factor" (TF). TF is a cell membrane protein that binds factor VII/VIIa to produce the catalytically active complex (TF:VIIa). A blood clot follows FXa-mediated activation of prothrombin. Blood clotting can be minimized by inactivation of TF to non-native forms which cannot optimally produce the TF:VIIa complex. Formation of FXa is believed to contribute to various thromboses including restenosis.

Thrombosis may be associated with invasive medical procedures such as cardiac surgery (e.g. angioplasty), abdominothoracic surgery, arterial surgery, deployment of an implementation (e.g., a stent or catheter), or endarterectomy. Further, thrombosis may accompany various thromboembolic disorders and coagulopathies such as a pulmonary embolism (e.g., atrial fibrillation with embolization) and disseminated intravascular coagulation, respectively. Manipulation of body fluids can also result in an undesirable thrombus, particularly in blood transfusions or fluid sampling, as well as procedures involving extracorporeal circulation (e.g., cardiopulmonary bypass surgery) and dialysis.

Anti-coagulants are frequently used to alleviate or avoid blood clots associated with thrombosis. Blood clotting often can be minimized or eliminated by administering a suitable anti-coagulant or mixture thereof, including one or more of a coumarin derivative (e.g., warfin and dicumarol) or a charged polymer (e.g., heparin, hirudin or hirulog). See e.g., Gilman et al., *supra*, R.J. Beigering et al., *Ann. Hemathol.*, **72**:177 (1996); J.D. Willerson, *Circulation*, **94**:866 (1996).

However, use of anti-coagulants is often associated with side effects such as hemorrhaging, re-occlusion, "white-clot" syndrome, irritation, birth defects, thrombocytopenia and hepatic dysfunction. Long-term administration of anti-coagulants can particularly increase risk of life-threatening illness (see e.g.,

Gilman et al., *supra*).

Certain antibodies with anti-platelet activity have also been used to alleviate various thromboses. For example, ReoPro™ is a therapeutic antibody that is routinely administered to alleviate various thromboembolic disorders such as those arising from angioplasty, myocardial infarction, unstable angina and coronary artery stenoses. Additionally, ReoPro™ can be used as a prophylactic to reduce the risk of myocardial infarction and angina (J.T. Willerson, *Circulation*, **94**:866 (1996); M.L. Simmons et al., *Circulation*, **89**:596 (1994)).

Certain anti-coagulant antibodies are also known. Particularly, certain TF-binding antibodies have been reported to inhibit blood coagulation, presumably by interfering with assembly of a catalytically active TF:VIIa complex (see e.g., Jeske et al., *SEM in THROM. and HEMO*, **22**:213 (1996); Ragni et al., *Circulation*, **93**:1913 (1996); European Patent No. 0 420 937 B1; W. Ruf et al., *Throm. Haemosp.*, **66**:529 (1991); M.M. Fiorie et al., *Blood*, **8**:3127 (1992)).

However, current TF-binding antibodies exhibit significant disadvantages which can minimize their suitably as anti-coagulants. For example, current TF-binding antibodies do not exhibit sufficient binding affinity for optimal anti-coagulant activity. Accordingly, for many thrombotic conditions, to compensate for such ineffective binding affinities, unacceptably high antibody levels must be administered to minimize blood coagulation. Further, current TF-binding antibodies do not effectively discriminate between native TF and non-native forms of TF, i.e. the current antibodies do not exhibit sufficient binding specificity. Still further, current TF-binding antibodies can not prevent FX from binding to TF and/or TF:VIIa complex.

It would thus be desirable to have an anti-coagulant antibody that binds native human TF with high affinity and selectivity to thereby inhibit undesired blood coagulation and the formation of blood clots. It would be further desirable to have such an anti-coagulant antibody that prevents the binding of Factor X to

TF/VIIa complex.

SUMMARY OF THE INVENTION

We have now discovered antibodies that provide superior anti-coagulant activity by binding native human TF with high affinity and specificity. Antibodies of the invention can effectively inhibit blood coagulation *in vivo*. Antibodies of the invention can bind native human TF, either alone or present in a TF:VIIa complex, effectively preventing factor X binding to TF or that complex, and thereby reducing blood coagulation.

Preferred antibodies of the invention are monoclonal and specifically bind a conformational epitope predominant to native human TF, which epitope provides an unexpectedly strong antibody binding site. Indeed, preferred antibodies of the invention bind to native human TF at least about 5 times greater, more typically at least about ten times greater than the binding affinity exhibited by prior anti-coagulant antibodies. Additionally, preferred antibodies of the invention are selective for native human TF, and do not substantially bind non-native or denatured TF. H36.D2.B7 (secreted by hybridoma ATCC HB-12255) is an especially preferred antibody of the invention.

Preferred antibodies of the invention bind TF so that FX does not effectively bind to the TF/factor VIIa complex whereby FX is not effectively converted to its activated form (FXa). Preferred antibodies of the invention can inhibit TF function by effectively blocking FX binding or access to TF molecules. See, for instance, the results of Example 3 which follows.

Preferred antibodies of the invention also do not significantly inhibit the interaction or binding between TF and factor VIIa, or inhibit activity of a TF:factor VIIa complex with respect to materials other than FX. See, for instance, the results of Example 4 which follows.

The invention also provides nucleic acids that encode antibodies of the invention. Nucleic acid and amino acid sequences (SEQ ID:NOS 1-4) of variable regions of H36.D2.B7 are set forth in Figures 1A and 1B of the drawings.

In preferred aspects, the invention provides methods for inhibiting blood coagulation and blood clot formation, and methods for reducing human TF levels.

In general, antibodies of the invention will be useful to modulate virtually any biological response mediated by FX binding to TF or the TF:VIIa complex, including blood coagulation as discussed above, inflammation and other disorders.

Antibodies of the invention are particularly useful to alleviate various thromboses, particularly to prevent or inhibit restenosis, or other thromboses following an invasive medical procedure such as arterial or cardiac surgery (e.g., angioplasty). Antibodies of the invention also can be employed to reduce or even effectively eliminate blood coagulation arising from use of medical implementation (e.g., a catheter, stent or other medical device). Preferred antibodies of the invention will be compatible with many anti-coagulant, anti-platelet and thrombolytic compositions, thereby allowing administration in a cocktail format to boost or prolong inhibition of blood coagulation.

Antibodies of the invention also can be employed as an anti-coagulant in extracorporeal circulation of a mammal, particularly a human subject. In such methods, one or more antibodies of the invention is administered to the mammal in an amount sufficient to inhibit blood coagulation prior to or during extracorporeal circulation such as may be occur with cardiopulmonary bypass surgery, organ transplant surgery or other prolonged surgeries.

Antibodies of the invention also can be used as a carrier for drugs, particularly pharmaceuticals targeted for interaction with a blood clot such as strepokinase, tissue plasminogen activator (t-PA) or urokinase. Similarly, antibodies of the invention can be used as a cytotoxic agent by conjugating a

suitable toxin to the antibody. Conjugates of antibodies of the invention also can be used to reduce tissue factor levels in a mammal, particularly a human, by administering to the mammal an effective amount of an antibody of the invention which is covalently linked a cell toxin or an effector molecule to provide complement-fixing ability and antibody-dependent cell-mediated cytotoxicity, whereby the antibody conjugate contacts cells expressing tissue factor to thereby reduce tissue factor levels in the mammal.

Antibodies of the invention also can be employed in *in vivo* diagnostic methods including *in vivo* diagnostic imaging of native human TF.

Antibodies of the invention also can be used in *in vitro* assays to detect native TF in a biological sample including a body fluid (e.g., plasma or serum) or tissue (e.g., a biopsy sample). More particularly, various heterogeneous and homogeneous immunoassays can be employed in a competitive or non-competitive format to detect the presence and preferably an amount of native TF in the biological sample.

Such assays of the invention are highly useful to determine the presence or likelihood of a patient having a blood coagulation or a blood clot. That is, blood coagulation is usually accompanied by TF expression on cells surfaces such as cells lining the vasculature. In the absence of blood coagulation, TF is not usually expressed. Thus, the detection of TF in a body fluid sample by an assay of the invention will be indicative of blood coagulation.

Antibodies of the invention also can be used to prepare substantially pure native TF, particularly native human TF, from a biological sample. Antibodies of the invention also can be used for detecting and purifying cells which express native TF.

Antibodies of the invention also can be employed as a component of a

diagnostic kit, e.g. for detecting and preferably quantitating native TF in a biological sample. Other aspects of the invention are discussed *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B shows the nucleic acid (SEQ ID NOS:1 and 3) and amino acid (SEQ ID NOS:2 and 4) sequences of light chain and heavy chain variable regions of H36.D2.B7 with hypervariable regions (CDRs or Complementarity Determining Regions) underlined (single underline for nucleic acid sequences and double underline for amino acid sequences).

Fig. 2 shows association (K_a) and disassociation (K_d) constants of anti-tissue factor antibodies as determined by ELISA or BIACore analysis.

Fig. 3 shows inhibition of TF-FVIIa complex mediated FX activation by pre-incubation with anti-tissue factor antibodies.

Fig. 4 shows inhibition of TF-FVIIa activity toward the FVIIa-specific substrate S-2288 by anti-tissue factor antibodies.

Fig. 5 shows the capacity of the H36 antibody to increase prothrombin time (PT) in a TF-initiated coagulation assay.

Figs. 6A and 6B graphically show the relationship between FXa formation and molar ratio of the H36.D2 antibody and rHTF. Fig. 6A: H36.D2 was pre-incubated with the TF-FVIIa complex prior to adding FX. Fig. 6B: H36.D2, TF-FVIIa and FX were added simultaneously.

Fig. 7 shows inhibition of TF-FVIIa activity by the H36.D2 antibody in a J-82 cell activation assay.

Figs. 8A and 8B are representations of dot blots showing that the H36.D2 antibody binds a conformational epitope on rhTF. Lane 1- native rHTF, Lane 2-

native rhTF treated with 8M urea, Lane 3- native rHTF treated with 8M urea and 5mM DTT. In Fig. 8A, the blot was exposed for approximately 40 seconds, whereas in Fig. 8B, the blot was exposed for 120 seconds.

DETAILED DESCRIPTION OF THE INVENTION

As discussed above, preferred antibodies of the invention exhibit substantial affinity for native human TF. In particular, preferred antibodies of the invention exhibit an association constant (K_a, M^{-1}) for native human TF of at least about 1×10^8 as determined by surface plasmon analysis (particularly, BIACore analysis in accordance with the procedures of Example 1 which follows), more preferably at least about 5×10^8 as determined by surface plasmon analysis, still more preferably a K_a (K_a, M^{-1}) for native human TF of at least about 1×10^{10} as determined by surface plasmon analysis. Such substantial binding affinity of antibodies of the invention contrast sharply from much lower binding affinities of previously reported antibodies.

In this regard, a quite low effective concentration of an antibody of the invention can be employed, e.g. a relatively low concentration of antibody can be employed to inhibit TF function as desired (e.g. at least about 95, 98 or 99 percent inhibition) in an *in vitro* assay such as described in Example 3 which follows.

The preferred antibodies are also highly specific for native human TF, and preferably do not substantially bind with non-native TF. Preferred antibodies do not substantially bind non-native TF or other immunologically unrelated molecules as determined, e.g. by standard dot blot assay (e.g. no or essentially no binding to non-native TF visually detected by such dot blot assay). References herein to "non-native TF" mean a naturally-occurring or recombinant human TF that has been treated with a choatropic agent so that the TF is denatured. Typical choatropic agents include a detergent (e.g. SDS), urea combined with dithiothreotol or β-mercaptoethanol; guanidine hydrochloride and the like. The H36, H36.D2 or H36.D2.B7 antibody does not substantially bind to such non-native TF. See, for instance, the results of Example 8 which follows and is a dot

blot assay.

As discussed above, preferred antibodies of the invention also bind with TF so that FX does not effectively bind to the TF/factor VIIa complex whereby FX is not effectively converted to its activated form (FXa). Particularly preferred antibodies of the invention exhibit will strongly inhibit FX activity to a TF/factor VIIa complex, e.g. an inhibition of at least about 50%, more preferably at least about 80%, and even more preferably at least about 90% or 95%, even at low TF concentrations such as less than about 1.0 nM TF, or even less than about 0.20 nM or 0.10 nM TF, as determined by a standard *in vitro* binding assay such as that of Example 3 which follows and includes contacting FX with a TF:factor VIIa complex both in the presence (i.e. experimental sample) and absence (i.e. control sample) of an antibody of the invention and determining the percent difference of conversion of FX to FXa between the experimental and control samples.

Antibodies of the invention are preferably substantially pure when used in the disclosed methods and assays. References to an antibody being "substantially pure" mean an antibody or protein which has been separated from components which naturally accompany it. For example, by using standard immunoaffinity or protein A affinity purification techniques, an antibody of the invention can be purified from a hybridoma culture by using native TF as an antigen or protein A resin. Similarly, native TF can be obtained in substantially pure form by using an antibody of the invention with standard immunoaffinity purification techniques. Particularly, an antibody or protein is substantially pure when at least 50% of the total protein (weight % of total protein in a given sample) is an antibody or protein of the invention. Preferably the antibody or protein is at least 60 weight % of the total protein, more preferably at least 75 weight %, even more preferably at least 90 weight %, and most preferably at least 98 weight % of the total material. Purity can be readily assayed by known methods such as SDS (PAGE) gel electrophoresis, column chromatography (e.g., affinity chromatography) or HPLC analysis.

The nucleic acid (SEQ ID NOS: 1 and 3) and amino acid (SEQ ID NOS: 2 and 4) sequences of a preferred antibody of the invention (H36.D2.B7) are shown in Figures 1A and 1B of the drawings. SEQ ID NOS. 1 and 2 are the nucleic acid and amino acid respectively of the light chain variable region, and SEQ ID NOS. 3 and 4 are the nucleic acid and amino acid respectively of the heavy chain variable region, with hypervariable regions (CDRs or Complementarity Determining Regions) underlined in all of those sequences.

Additional preferred antibodies of the invention will have substantial sequence identity to either one or both of the light chain or heavy sequences shown in Figures 1A and 1B. More particularly, preferred antibodies include those that have at least about 70 percent homology (sequence identity) to SEQ ID NOS. 2 and/or 4, more preferably about 80 percent or more homology to SEQ ID NOS. 2 and/or 4, still more preferably about 85, 90 or 95 percent or more homology to SEQ ID NOS. 2 and/or 4.

Preferred antibodies of the invention will have high sequence identity to hypervariable regions (shown with double underlining in Figures 1A and 1B) of SEQ ID NOS. 2 and 4). Especially preferred antibodies of the invention will have one, two or three hypervariable regions of a light chain variable region that have high sequence identity (at least 90% or 95% sequence identity) to or be the same as one, two or three of the corresponding hypervariable regions of the light chain variable region of H36.D2.B7 (those hypervariable regions shown with underlining in Figure 1A and are the following: 1) LASQTID (SEQ ID NO:5); 2) AATNLAD (SEQ ID NO:6); and 3) QQVYSSPFT (SEQ ID NO:7)).

Especially preferred antibodies of the invention also will have one, two or three hypervariable regions of a heavy chain variable region that have high sequence identity (at least 90% or 95% sequence identity) to or be the same as one, two or three of the corresponding hypervariable regions of the heavy chain variable region of H36.D2.B7 (those hypervariable regions shown with underlining in Figure 1B and are the following: 1) TDYNVY (SEQ ID NO:8); 2)

YIDPYNGITIYDQNFKG (SEQ ID NO:9); and 3) DVTTALDF (SEQ ID NO:10).

Nucleic acids of the invention preferably are of a length sufficient (preferably at least about 100, 200 or 250 base pairs) to bind to the sequence of SEQ ID NO:1 and/or SEQ ID NO:3 under the following moderately stringent conditions (referred to herein as "normal stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C.

More preferably, nucleic acids of the invention (preferably at least about 100, 200 or 250 base pairs) will bind to the sequence of SEQ ID NO:1 and/or SEQ ID NO:3 under the following highly stringent conditions (referred to herein as "high stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M sodium citrate (SSC) buffer at a temperature of 42°C and remaining bound when subject to washing twice with that SSC buffer at 42°C.

Nucleic acids of the invention preferably comprise at least 20 base pairs, more preferably at least about 50 base pairs, and still more preferably a nucleic acid of the invention comprises at least about 100, 200, 250 or 300 base pairs.

Generally preferred nucleic acids of the invention will express an antibody of the invention that exhibits the preferred binding affinities and other properties as disclosed herein.

Preferred nucleic acids of the invention also will have substantial sequence identity to either one or both of the light chain or heavy sequences shown in Figures 1A and 1B. More particularly, preferred nucleic acids will comprise a sequence that has at least about 70 percent homology (sequence identity) to SEQ ID NOS. 1 and/or 3, more preferably about 80 percent or more homology to SEQ

ID NOS. 1 and/or 3, still more preferably about 85, 90 or 95 percent or more homology to SEQ ID NOS. 1 and/or 3.

Particularly preferred nucleic acid sequences of the invention will have high sequence identity to hypervariable regions (shown with underlining in Figures 1A and 1B) of SEQ ID NOS. 1 and 3). Especially preferred nucleic acids include those that code for an antibody light chain variable region and have one, two or three sequences that code for hypervariable regions and have high sequence identity (at least 90% or 95% sequence identity) to or be the same as one, two or three of the sequences coding for corresponding hypervariable regions of H36.D2.B7 (those hypervariable regions shown with underlining in Figure 1A and are the following: 1) CTGGCAAGTCAGACCATTGAT (SEQ ID NO:11); 2) GCTGCCACC
AACTTGGCAGAT (SEQ ID NO:12); and 3) CAACAAGTTTACAGTTCT
CCATTCACGT (SEQ ID NO:13)).

Especially preferred nucleic acids also code for an antibody heavy chain variable region and have one, two or three sequences that code for hypervariable regions and have high sequence identity (at least 90% or 95% sequence identity) to or be the same as one, two or three of the sequences coding for corresponding hypervariable regions of H36.D2.B7 (those hypervariable regions shown with underlining in Figure 1B and are the following: 1) ACTGACTACAACGTGTAC (SEQ ID NO:14); 2) TATATTGAT
CCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGC (SEQ ID NO:15); and 3) GATGTGACTACGGCCCTTGACTTC (SEQ ID NO:16)).

Nucleic acids of the invention are isolated, usually constitutes at least about 0.5%, preferably at least about 2%, and more preferably at least about 5% by weight of total nucleic acid present in a given fraction. A partially pure nucleic acid constitutes at least about 10%, preferably at least about 30%, and more preferably at least about 60% by weight of total nucleic acid present in a given fraction. A pure nucleic acid constitutes at least about 80%, preferably at least

about 90%, and more preferably at least about 95% by weight of total nucleic acid present in a given fraction.

Antibodies of the invention can be prepared by techniques generally known in the art, and are typically generated to a purified sample of native TF, typically native human TF, preferably purified recombinant human tissue factor (rhTF). Truncated recombinant human tissue factor or "rhTF" (composed of 243 amino acids and lacking the cytoplasmic domain) is particularly preferred to generate antibodies of the invention. The antibodies also can be generated from an immunogenic peptide that comprises one or more epitopes of native TF that are not exhibited by non-native TF. References herein to "native TF" include such TF samples, including such rhTF. As discussed above, monoclonal antibodies are generally preferred, although polyclonal antibodies also can be employed.

More particularly, antibodies can be prepared by immunizing a mammal with a purified sample of native human TF, or an immunogenic peptide as discussed above, alone or complexed with a carrier. Suitable mammals include typical laboratory animals such as sheep, goats, rabbits, guinea pigs, rats and mice. Rats and mice, especially mice, are preferred for obtaining monoclonal antibodies. The antigen can be administered to the mammal by any of a number of suitable routes such as subcutaneous, intraperitoneal, intravenous, intramuscular or intracutaneous injection. The optimal immunizing interval, immunizing dose, etc. can vary within relatively wide ranges and can be determined empirically based on this disclosure. Typical procedures involve injection of the antigen several times over a number of months. Antibodies are collected from serum of the immunized animal by standard techniques and screened to find antibodies specific for native human TF. Monoclonal antibodies can be produced in cells which produce antibodies and those cells used to generate monoclonal antibodies by using standard fusion techniques for forming hybridoma cells. See G. Kohler, et al., *Nature*, **256**:456 (1975). Typically this involves fusing an antibody producing cell with an immortal cell line such as a myeloma cell to produce the hybrid cell. Alternatively, monoclonal antibodies can be

produced from cells by the method of Huse, et al., *Science*, **256**:1275 (1989).

One suitable protocol provides for intraperitoneal immunization of a mouse with a composition comprising purified rhTF complex conducted over a period of about two to seven months. Spleen cells then can be removed from the immunized mouse. Sera from the immunized mouse is assayed for titers of antibodies specific for rhTF prior to excision of spleen cells. The excised mouse spleen cells are then fused to an appropriate homogenic or heterogenic (preferably homogenic) lymphoid cell line having a marker such as hypoxanthine-guanine phosphoribosyltransferase deficiency ($HGPRT^-$) or thymidine kinase deficiency (TK^-). Preferably a myeloma cell is employed as the lymphoid cell line. Myeloma cells and spleen cells are mixed together, e.g. at a ratio of about 1 to 4 myeloma cells to spleen cells. The cells can be fused by the polyethylene glycol (PEG) method. See G. Kohler, et al., *Nature, supra*. The thus cloned hybridoma is grown in a culture medium, e.g. RPMI-1640. See G. E. More, et al., *Journal of American Medical Association*, **199**:549 (1967). Hybridomas, grown after the fusion procedure, are screened such as by radioimmunoassay or enzyme immunoassay for secretion of antibodies that bind specifically to the purified rhTF, e.g. antibodies are selected that bind to the purified rhTF, but not to non-native TF. Preferably an ELISA is employed for the screen. Hybridomas that show positive results upon such screening can be expanded and cloned by limiting dilution method. Further screens are preferably performed to select antibodies that can bind to rhTF in solution as well as in a human fluid sample. The isolated antibodies can be further purified by any suitable immunological technique including affinity chromatography. A hybridoma culture producing the particular preferred H36.D2.B7 antibody has been deposited pursuant to the Budapest Treaty with the American Type Culture Collection (ATCC) at 12301 Parklawn Drive, Rockville, MD, 10852. The hybridoma culture was deposited with the ATCC on January 8, 1997 and was assigned Accession Number ATCC HB-12255.

For human therapeutic applications, it may be desirable to produce chimeric antibody derivatives, e.g. antibody molecules that combine a non-human

animal variable region and a human constant region, to thereby render the antibodies less immunogenic in a human subject than the corresponding non-chimeric antibody. A variety of types of such chimeric antibodies can be prepared, including e.g. by producing human variable region chimeras, in which parts of the variable regions, especially conserved regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. See also discussions of humanized chimeric antibodies and methods of producing same in S.L. Morrison, *Science*, **229**:1202-1207 (1985); Oi et al., *BioTechniques*, **4**:214 (1986); Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, **80**:7308-7312 (1983); Kozbor et al., *Immunology Today*, **4**:7279 (9183); Olsson et al., *Meth. Enzymol.*, **9**:3-16 (1982). Additionally, transgenic mice can be employed. For example, transgenic mice carrying human antibody repertoires have been created which can be immunized with native human TF. Splenocytes from such immunized transgenic mice can then be used to create hybridomas that secrete human monoclonal antibodies that specifically react with native human TF as described above. See N. Lonberg et al., Nature, **368**:856-859 (1994); L.L. Green et al., Nature Genet., **7**:13-21 (1994); S.L. Morrison, *Proc. Natl. Acad. Sci. U.S.A.*, **81**:6851-6855 (1994).

Nucleic acids of antibodies of the invention also can be prepared by polymerase chain reaction (see primers disclosed in Example 1 which follows). See generally, Sambrook et al., Molecular Cloning (2d ed. 1989). Such nucleic acids also can be synthesized by known methods, e.g. the phosphate triester method (see Oligonucleotide Synthesis, IRL Press (M.J. Gait, ed., 1984)), or by using a commercially available automated oligonucleotide synthesizer. Such a prepared nucleic acid of the invention can be employed to express an antibody of the invention by known techniques. For example, a nucleic acid coding for an antibody of the invention can be incorporated into a suitable vector by known methods such as by use of restriction enzymes to make cuts in the vector for insertion of the construct followed by ligation. The vector containing the inserted nucleic acid sequence, suitably operably linked to a promoter sequence, is then introduced into host cells for expression. See, generally, Sambrook et al., *supra*.

Selection of suitable vectors can be made empirically based on factors relating to the cloning protocol. For example, the vector should be compatible with, and have the proper replicon for the host cell that is employed. Further, the vector must be able to accommodate the inserted nucleic acid sequence. Suitable host cells will include a wide variety of eukaryotic or prokaryotic cells such as *E. coli* and the like.

The molecular weight of the antibodies of the invention will vary depending on several factors such as the intended use and whether the antibody includes a conjugated or recombinantly fused toxin, pharmaceutical, or detectable label or the like. In general, an antibody of the invention will have a molecular weight of between approximately 20 to 150kDa. Such molecular weights can be readily are determined by molecular sizing methods such as SDS-PAGE gel electrophoresis followed by protein staining or Western blot analysis.

"Antibody of the invention" or other similar term refers to whole immunoglobulin as well immunologically active fragments which bind native TF. The immunoglobulins and immunologically active fragments thereof include an antibody binding site (i.e., peritope capable of specifically binding native human TF). Exemplary antibody fragments include, for example, Fab, F(v), Fab', $F(ab')_2$ fragments, "half molecules" derived by reducing the disulfide bonds of immunoglobulins, single chain immunoglobulins, or other suitable antigen binding fragments (see e.g., Bird et al., *Science*, pp. 242-424 (1988); Huston et al., *PNAS, (USA)*, **85**:5879 (1988); Webber et al., *Mol. Immunol.*, **32**:249 (1995)). The antibody or immunologically active fragment thereof may be of animal (e.g., a rodent such as a mouse or a rat), or chimeric form (see Morrison et al., *PNAS*, **81**:6851 (1984); Jones et al., *Nature*, pp. 321, 522 (1986)). Single chain antibodies of the invention can be preferred.

Similarly, a "nucleic acid of the invention" refers to a sequence which can be expressed to provide an antibody of the invention as such term is specified to mean immediately above.

As discussed above, antibodies of the invention can be administered to a mammal, preferably a primate such as a human, to prevent or reduce thromboses such as restenosis, typically in a composition including one or more pharmaceutically acceptable non-toxic carriers such as sterile water or saline, glycols such as polyethylene glycol, oils of vegetable origin, and the like. In particular, biocompatible, biodegradable lactide polymer, lactide glycolide copolymer or polyoxyethylene, polyoxypropylene copolymers may be useful excipients to control the release of the antibody-containing compositions described herein. Other potentially useful administration systems include ethylene vinyl acetate copolymer particles, osmotic pumps, and implantable infusion systems and liposomes. Generally, an anti-coagulant composition of the invention will be in the form of a solution or suspension, and will preferably include approximately 0.01% to 10% (w/w) of the antibody of the present invention, preferably approximately 0.01% to 5% (w/w) of the antibody. The antibody can be administered as a sole active ingredient in the composition, or as a cocktail including one or more other anti-coagulant (e.g., heparin, hirudin, or hirulog), anti-platelet (e.g., ReoPro), or thrombolytic agents (e.g., tissue plasminogen activator, strepokinase and urokinase). Additionally, antibodies of the invention can be administered prior to, or after administration of one or more suitable anti-coagulant, anti-platelet or thrombolytic agents to boost or prolong desired anti-coagulation activity.

As also discussed above, antibodies of the invention can be employed to reduce potential blood coagulation arising from use of medical implementation, e.g. an indwelling device such as a catheter, stent, etc. In one preferred method, the implementation can be treated with an antibody of the invention (e.g., as a 1 mg/ml saline solution) prior to contact with a body fluid. Alternatively, or in addition, an antibody of the invention can be combined with the body fluid in an amount sufficient to minimize blood clotting.

Therapeutic anti-coagulant compositions according to the invention are

suitable for use in parenteral or intravenous administration, particularly in the form of liquid solutions. Such compositions may be conveniently administered in unit dose and may be prepared in accordance with methods known in the pharmaceutical art. See *Remington's Pharmaceutical Sciences*, (Mack Publishing Co., Easton PA, (1980)). By the term "unit dose" is meant a therapeutic composition of the present invention employed in a physically discrete unit suitable as unitary dosages for a primate such as a human, each unit containing a pre-determined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent or carrier. The unit dose will depend on a variety of factors including the type and severity of thrombosis to be treated, capacity of the subject's blood coagulation system to utilize the antibody, and degree of inhibition or neutralization of FX activation desired. Precise amounts of the antibody to be administered typically will be guided by judgement of the practitioner, however, the unit dose will generally depend on the route of administration and be in the range of 10 ng/kg body weight to 50 mg/kg body weight per day, more typically in the range of 100 ng/kg body weight to about 10 mg/kg body weight per day. Suitable regiments for initial administration in booster shots are also variable but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous or intermittent intravenous infusions may be made sufficient to maintain concentrations of at least from about 10 nanomolar to 10 micromolar of the antibody in the blood.

In some instances, it may be desirable to modify the antibody of the present invention to impart a desirable biological, chemical or physical property thereto. More particularly, it may be useful to conjugate (i.e. covalently link) the antibody to a pharmaceutical agent, e.g. a fibrinolytic drug such as t-PA, streptokinase, or urokinase to provide fibrinolytic activity. Such linkage can be accomplished by several methods including use of a linking molecule such as a heterobifunctional protein cross-linking agent, e.g. SPDP, carbodimide, or the like, or by recombinant methods.

In addition to pharmaceuticals such as a fibrinolytic agent, an antibody of the invention can be conjugated to a toxin of e.g. plant or bacterial origin such as diphtheria toxin (i.e., DT), shiga toxin, abrin, cholera toxin, ricin, saporin, pseudomonas exotoxin (PE), pokeweed antiviral protein, or gelonin. Biologically active fragments of such toxins are well known in the art and include, e.g., DT A chain and ricin A chain. The toxin can also be an agent active at cell surfaces such as phospholipases (e.g., phospholipase C). As another example, the toxin can be a chemotherapeutic drug such as, e.g., vendesine, vincristine, vinblastin, methotrexate, adriamycin, bleomycin, or cisplatin, or, the toxin can be a radionuclide such as, e.g., iodine-131, yttrium-90, rhenium-188 or bismuth-212 (see generally, Moskaug et al., *J. Biol. Chem.*, **264**:15709 (1989); I. Pastan et al., *Cell*, **47**:641 (1986); Pastan et al., *Recombinant Toxins as Novel Therapeutic Agents*, *Ann. Rev. Biochem.*, **61**:331 (1992); *Chimeric Toxins Olsnes and Phil, Pharmac. Ther.*, **25**:355 (1982); published PCT Application No. WO 94/29350; published PCT Application No. WO 94/04689; and U.S. Patent No. 5,620,939). Also, as discussed above, in addition to a toxin, an antibody of the invention can be conjugated to an effector molecule (e.g. IgG1 or IgG3) to provide complement-fixing ability and antibody-dependent cell-mediated cytoxicity upon administration to a mammal.

Such an antibody/cytotoxin or effector molecule conjugate can be administered in a therapeutically effective amount to a mammal, preferably a primate such as a human, where the mammal is known to have or is suspected of having tumor cells, immune system cells, or endothelia capable of expressing TF. Exemplary of such tumor cells, immune system cells and endothelia include malignancies of the breast and lung, monocytes and vascular endothelia.

Antibodies of the invention also can be conjugated to a variety of other pharmaceutical agents in addition to those described above such as, e.g., drugs, enzymes, hormones, chelating agents capable of binding a radionuclide, as well as other proteins and polypeptides useful for diagnosis or treatment of disease. For diagnostic purposes, the antibody of the present invention can be used either

detectably-labelled or unlabelled. For example, a wide variety of labels may be suitably employed to detectably-label the antibody, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands such as, e.g., haptens, and the like.

Diagnostic methods are also provided including *in vivo* diagnostic imaging [see, e.g., A.K. Abbas, *Cellular and Molecular Immunology*, pg. 328 (W.B. Saunders Co. 1991)]. For most *in vivo* imaging applications, an antibody of the invention can be detectably-labeled with, e.g., ^{125}I, ^{32}P, ^{99}Tc, or other detectable tag, and subsequently administered to a mammal, particularly a human, for a pre-determined amount of time sufficient to allow the antibody to contact a desired target. The subject is then scanned by known procedures such as scintigraphic camera analysis to detect binding of the antibody. The analysis could aid in the diagnosis and treatment of a number of thromboses such as those specifically disclosed herein. The method is particularly useful when employed in conjunction with cardiac surgery, particularly angioplasty, or other surgical procedure where undesired formation of a blood clot can occur, to visualize the development or movement of a blood clot.

Antibodies of the invention also can be used to prepare substantially pure (e.g., at least about 90% pure, preferably at least about 96 or 97% pure) native TF, particularly native human TF from a biological sample. For example, native TF can be obtained as previously described (see e.g., L.V.M. Rao et al., *Thrombosis Res.*, **56**:109 (1989)) and purified by admixing the solution with a solid support comprising the antibody to form a coupling reaction admixture. Exemplary solid supports include a wall of a plate such as a microtitre plate, as well as supports including or consisting of polystyrene, polyvinylchloride, a cross-linked dextran such as Sephadex™ (Pharmacia Fine Chemicals), agarose, polystyrene beads (Abbott Laboratories), polyvinyl chloride, polystyrene, polyacrylmide in cross-linked form, nitrocellulose or nylon and the like. The TF can then be isolated from the solid support in substantially pure form in accordance with standard immunological techniques. See generally Harlow and Lane *supra* and Ausubel et

al. *supra*).

As also discussed above, antibodies of the invention can be employed to detect native human TF in a biological sample, particularly native TF associated with a blood clot. Exemplary biological samples include blood plasma, serum, saliva, urine, stool, vaginal secretions, bile, lymph, ocular humors, cerebrospinal fluid, cell culture media, and tissue, particularly vascular tissues such as cardiac tissue. Samples may be suitably obtained from a mammal suffering from or suspected of suffering from a thrombosis, preferably restenosis, associated with, e.g., an invasive medical procedure such as cardiopulmonary bypass surgery; a heart ailment such as myocardial infarction, cardiomyopathy, valvular heart disease, unstable angina, or artrial fibrillation associated with embolization; a coagulopathy including disseminated intravascular coagulation, deployment of an implementation such as a stent or catheter; shock (e.g., septic shock syndrome), vascular trauma, liver disease, heat stroke, malignancies (e.g., pancreatic, ovarian, or small lung cell carcinoma), lupus, eclampsia, perivascular occlusive disease, and renal disease.

For such assays, an antibody of the invention can be detectably-labelled with a suitable atom or molecule e.g., radioactive iodine, tritium, biotin, or reagent capable of generating a detectable product such as an anti-iodiotypic antibody attached to an enzyme such as β-galactosidase or horseradish peroxidase, or a fluorescent tag (e.g., fluorescein or rhodamine) in accordance with known methods. After contacting the biological sample with the detectably-labelled antibody, any unreacted antibody can be separated from the biological sample, the label (or product) is detected by conventional immunological methods including antibody capture assay, antibody sandwich assay, RIA, ELISA, immunoprecipitation, immunoabsorption and the like (see Harlow and Lane, *supra*; Ausubel et al. *supra*). Any label (or product) in excess of that detected in a suitable control sample is indicative of the presence of native TF, more particularly a blood clot, in the biological sample. For example, antibodies of the invention can be detectably-labelled to detect, and preferably quantitate, native TF

in accordance with standard immunological techniques such as antibody capture assay, ELISA, antibody sandwich assay, RIA, immunoprecipitation, immunoabsorption and the like. In some cases, particularly when a tissue is used, the immunological technique may include tissue fixation with a reagent known to substantially maintain protein conformation (e.g., dilute formaldehyde). See generally, Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, (1989); Harlow and Lane in *Antibodies: A Laboratory Manual*, CSH Publications, NY (1988).

Antibodies of the invention also can be used for detecting and purifying cells which express native TF, including fibroblasts, brain cells, immune cells, (e.g., monocytes), epithelia, as well as certain malignant cells. Preferred methods of detecting and purifying the cells include conventional immunological methods (e.g., flow cytometry methods such as FACS, and immunopanning). Substantially pure populations of cells expressing native TF are useful in clinical and research settings, e.g., to establish such cells as cultured cells for screening TF-binding antibodies.

The invention also provides test and diagnostic kits for detection of native TF, particularly native human TF, in a test sample, especially a body fluid such as blood, plasma, etc., or tissue as discussed above. A preferred kit includes a detectably-labelled antibody of the invention. The diagnostic kit can be used in any acceptable immunological format such as an ELISA format to detect the presence or quantity of native TF in the biological sample.

All documents mentioned herein are fully incorporated by reference in their entirety.

The following non-limiting examples are illustrative of the invention. In the following examples and elsewhere the antibodies H36 and H36.D2 are referred to. Those antibodies are the same antibody as H36.D2.B7, but H36 is derived from the mother clone, and H36.D2 is obtained from the primary clone,

whereas H36.D2.B7 is obtained from the secondary clone. No differences have been observed between those three clones with respect to ability to inhibit TF or other physical properties.

EXAMPLE 1 - Preparation and Cloning of Anti-rhTF Monoclonal Antibodies

Monoclonal antibodies against rhTF were prepared as follows.

A. Immunization and Boosts

Five female BALB/c mice were immunized with 10 μg each of lipidated, purified rhTF. The mice were initially sensitized intraperitoneally using Hunter's Titermax adjuvant. Three final boosts were administered in 0.85% NaCl. Boosts were 2, 5.5, and 6.5 months post initial sensitization. All boosts were given intraperitoneally, except the first which was subcutaneous. The final boost was given 3 days pre-fusion and 20 μg was administered.

B. Fusion of Mouse Spleen Lymphocytes with Mouse Myeloma Cells

Lymphocytes from the spleen of one rhTF immunized BALB/c mouse was fused to X63-Ag8.653 mouse myeloma cells using PEG 1500. Following exposure to the PEG, the cells were incubated for one hour in heat inactivated fetal bovine serum at 37°C. The fused cells were then resuspended in RPMI 1640 and incubated overnight at 37°C with 10% CO_2. The cells were plated the next day using RPMI 1640 and supplemented with macrophage culture supernatant.

C. ELISA Development

Plates for the ELISA assay were coated with 100 microliters of recombinant tissue factor (0.25 μg/ml) in a carbonate based buffer. All steps were performed at room temperature. Plates were blocked with BSA, washed, and then the test samples and controls were added. Antigen/antibody binding was detected by incubating the plate with goat anti-mouse HRP conjugate (Jackson ImmunoResearch Laboratories) and then using an ABTS peroxidase substrate system (Kirkegaad and Perry Laboratories). Absorbance was read on an automatic

plate reader at a wavelength of 405 nm.

D. Stabilization of rhTF Hybridoma Cell Lines

Two weeks after fusion, screening of hybridoma colonies by specific rhTF ELISA was started. Screening for new colonies continued for three weeks. The positive clones were tested every one to two weeks for continued antibody production until fifteen stable clones were isolated.

E. Primary and Secondary Cloning

Limiting dilution cloning was performed on each of the positive stable hybridomas to obtain primary clones. The cells were thawed, grown in culture for a short period of time, and then diluted from 10 cells/well to 0.1 cells/well. Primary clones were tested by anti-rhTF ELISA and five to six positive clones were expanded and frozen.

Secondary clone of anti-rhTF antibody, H36.D2.B7, was obtained from primary clone, H36.D2, prepared and stored in liquid nitrogen as described above. Four different dilutions, 5 cells/well, 2 cells/well, 1 cell/well, 0.5 cells/well of the primary clone were prepared in 96-wells microtiter plates to start the secondary cloning. Cells were diluted in IMDM tissue culture media containing the following additives: 20% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 1% GMS-S, 0.075% $NaHCO_3$. To determine clones that secrete anti-rhTF antibody, supernatants from five individual wells of the 0.2 cells/well microtiter plate were withdrawn after two weeks of growth and tested for the presence of anti-rhTF antibody by ELISA assays as described above. All five clones showed positive results in the ELISA assay, with H36.D2.B7 being the best antibody producer. All five clones were adapted and expanded in RPMI media containing the following additive: 10% FBS, 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 1% GMS-S, 0.075% $NaHCO_3$, and 0.013 mg/ml of oxalaacetic acid. H36.D2.B7 was purified by Protein A affinity chromatography from the supernatant of cell culture and was tested for its ability to inhibit TF:VIIa in a FX activation assay.

The results indicated that H36.D2.B7 had the same inhibition as H36.D2 antibody. All cells were stored in liquid nitrogen.

F. Isolation of total RNA from H36.D2.B7

269 μg of total RNA was isolated from 2.7 x 10^5 H36.D2.B7 hybridoma cells. The isolation of total RNA was performed as described in the RNeasy Midi Kits protocol from Qiagen. The RNA sample was stored in water at -20°C until needed.

G. cDNA Synthesis and Cloning of Variable Regions of H36.D2.B7 Gene

To obtain the first strand of cDNA, a reaction mixture containing 5 μg of total RNA isolated as above, back primers JS300 (all primers are identified below) for the heavy chain (HC) and OKA 57 for the light chain (LC), RNase inhibitor, dNTP's, DTT, and superscript II reverse transcriptase, was prepared and incubated at 42°C for 1 hour. The reaction tube is then incubated at 65°C for 15 minutes to stop the transcription. After cooling down, five units of RNase H was then added and the reaction was allowed to incubate at 37°C for 20 minutes. The cDNA sample was stored at -70°C until needed.

PCR (polymerase chain reaction) was conducted separately to clone the variable regions of both HC and LC of anti-rhTF, H36.D2.B7 from the cDNA made as above (nucleic acid and amino acid sequences of those HC and LC variable regions set forth in Figs. 1A and 1B). Three rounds of PCR were conducted. Round 1: PCR was run for 35 cycles at 96°C, 53°C and 72°C using front primer JS002 and back primer JS300 for HC. For LC front primer JS009 and back primer OKA 57 were used and PCR was run for 35 cycles at 96°C, 63°C and 72°C. Round 2: PCR of both HC and LC was run the same as in Round 1 with the exception that pMC-18 was used for HC front primer and pMC-15 for LC front primer. Round 3: PCR was run for 30 cycles at 96°C, 60-65°C and 72°C using H36HCF and H36HCR primers for HC. For LC, PCR was run for 30 cycles at 96°C, 58°C and 72°C using H36LCF and H36LCR primers.

The following primers were used for cloning H36.D2.B7 variable regions of HC and LC.

OKA 57:

5'-GCACCTCCAGATGTTAACTGCTC-3' (SEQ ID NO: 17)

JS300:

5'-GAARTAVCCCTTGACCAGGC-3' (SEQ ID NO: 18)

JS009:

5'-GGAGGCGGCGGTTCTGACATTGTGMTGWCMCARTC-3' (SEQ ID NO: 19)

JS002:

5'-ATTTCAGGCCCAGCCGGCCATGGCCGARGTYCARCTKCARCARYC-3' (SEQ ID NO: 20)

pMC-15:

5'-CCCGGGCCACCATGKCCCCWRCTCAGYTYCTKG-3' (SEQ ID NO: 21)

pMC-18:

5'-CCCGGGCCACCATGGRATGSAGCTGKGTMATSCTC-3' (SEQ ID NO: 22)

H36HCF:

5'-ATATACTCGCGACAGCTACAGGTGTCCACTCCGAGATCCAGCTGCAGCAGTC-3' (SEQ ID NO: 23)

H36HCR:

5'-GACCTGAATTCTAAGGAGACTGTGAGAGTGG-3' (SEQ ID NO: 24)

H36LCF:

5'-TTAATTGATATCCAGATGACCCAGTCTCC-3' (SEQ ID NO: 25)

H36LCR:
TAATCGTTCGAAAAGTGTACTTACGTTTCAGCTCCAGCTTGGTCC
(SEQ ID NO: 26)

where in the above SEQ ID NOS: 17 through 26: K is G or T; M is A or C; R is A or G; S is C or G; V is A, C or G; W is A or T; Y is C or T.

EXAMPLE 2 - Binding activity of Mabs of the invention

Mabs of the invention as prepared in Example 1 above were employed. The rhTF molecule was expressed in *E.coli* and purified by immunoaffinity chromatography in accordance with standard methods (see Harlow and Lane, *supra*, Ausubel et al. *supra*). Mab association (K_a) and dissociation (K_d) constants were determined by ELISA and surface plasmon resonance (i.e., BIACore) assays (see e.g., Harlow and Lane, *supra*; Ausubel et al. *supra*; Altschuh et al., *Biochem.*, **31**:6298 (1992); and the BIAcore method disclosed by Pharmacia Biosensor). For BIACore assays, rhTF was immobilized on a biosensor chip in accordance with the manufacturer's instructions. Constants for each Mab were determined at four antibody concentrations (0.125 nM, 0.25 nM, 0.5 nM, and 1 nM).

Protein concentrations were determined by standard assay (M.M. Bradford, *Anal. Biochem.*, **72**:248 (1976)) using Bovine Serum Albumin as a standard and a commercially available dye reagent (Bio-Rad).

Fig. 2 shows association and disassociation constants for each anti-rhTF Mab. Mab H36 exhibited the highest association rate (K_a= 3.1 X 10^{10} M^{-1}) and the lowest disassociation rate (K_d=3.2 X 10^{-11} M) of any of the anti-rhTF Mabs tested.

EXAMPLE 3 - FXa-specific Substrate Assay

In general, the experiments described herein were conducted using rhTF lipidated with phosphatidycholine (0.07 mg/ml) and phosphatidylserine (0.03

mg/ml) at a 70/30 w/w ratio in 50 mM Tris-HCl, pH 7.5, 0.1% bovine serum albumin (BSA) for 30 minutes at 37°C. A stock solution of preformed TF:VIIa complex was made by incubating 5 nM of the lipidated rhTF and 5 nM of FVIIa for 30 minutes at 37°C. The TF:VIIa complex was aliquoted and stored at -70°C until needed. Purified human factors VII, VIIa, and FX were obtained from Enyzme Research Laboratories, Inc. The following buffer was used for all FXa and FVIIa assays: 25 mM Hepes-NaOH, 5 mM $CaCl_2$, 150 mM NaCl, 0.1% BSA, pH 7.5.

Mabs were screened for capacity to block TF:VIIa-mediated activation of FX to FXa. The FX activation was determined in two discontinuous steps. In the first step (FX activation), FX conversion to FXa was assayed in the presence of Ca^{+2}. In the second step (FXa activity assay), FX activation was quenched by EDTA and the formation of FXa was determined using a FXa-specific chromogenic substrate (S-2222). The S-2222 and S-2288 (see below) chromogens were obtained from Chromogenix (distributed by Pharmacia Hepar Inc.). FX activation was conducted in 1.5 ml microfuge tubes by incubating the reaction with 0.08 nM TF:VIIa, either pre-incubated with an anti-rhTF antibody or a buffer control. The reaction was subsequently incubated for 30 minutes at 37°C, then 30 nM FX was added followed by an additional incubation for 10 minutes at 37°C. FXa activity was determined in 96-well titre plates. Twenty microlitres of sample was withdrawn from step one and admixed with an equal volume of EDTA (500 mM) in each well, followed by addition of 0.144 ml of buffer and 0.016 ml of 5 mM S-2222 substrate. The reaction was allowed to incubate for an additional 15-30 minutes at 37°C. Reactions were then quenched with 0.05 ml of 50% acetic acid, after which, absorbance at 405 nm was recorded for each reaction. The inhibition of TF:VIIa activity was calculated from OD_{405nm} values in the experimental (plus antibody) and control (no antibody) samples. In some experiments, an anti-hTF antibody, TF/VIIa, and FX were each added simultaneously to detect binding competition. Fig. 3 shows that the H36.D2 MAb (in bold) inhibited TF:/VIIa activity toward FX to a significantly greater extent (95%) than other anti-rHTF Mabs tested.

EXAMPLE 4 - FVIIa-Specific Substrate Assay

Mabs were further screened by an FVIIa specific assay. In this assay, 5 nM lipidated rhTF was first incubated with buffer (control) or 50 nM antibody (experimental) in a 96-well titre plate for 30 minutes at 37°C, then admixed with 5 nM purified human FVIIa (V_T= 0.192 ml), followed by 30 minutes incubation at 37°C. Eight microliters of a 20 mM stock solution of the FVIIa specific substrate S-2288 was then added to each well (final concentration, 0.8 mM). Subsequently, the reaction was incubated for one hour at 37°C. Absorbance at 405 nm was then measured after quenching with 0.06 ml of 50% acetic acid. Percent inhibition of TF/VIIa activity was calculated from OD_{405nm} values from the experimental and control samples.

Fig. 4 shows the H36 antibody did not significantly block TF/VIIa activity toward the S-2288 substrate when the antibody was either pre-incubated with TF (prior to VIIa addition) or added to TF pre-incubated with VIIa (prior to adding the antibody). This indicates that H36 does not interfere with the interaction (binding) between TF and FVIIa, and that H36 also does not inhibit TF:VIIa activity toward a peptide substrate.

EXAMPLE 5 - Prothrombin Time (PT) Assay

Calcified blood plasma will clot within a few seconds after addition of thromplastin (TF); a phenomenon called the "prothrombin time" (PT). A prolonged PT is typically a useful indicator of anti-coagulation activity (see e.g., Gilman et al. *supra*).

The H36.D2 antibody was investigated for capacity to affect PT according to standard methods using commercially available human plasma (Ci-Trol Control, Level I obtained from Baxter Diagnostics Inc.). Clot reactions were initiated by addition of lipidated rhTF in the presence of Ca^{++}. Clot time was monitored by an automated coagulation timer (MLA Electra 800). PT assays were initiated by injecting 0.2 ml of lipidated rhTF (in a buffer of 50 mM Tris-HCl, pH

7.5, containing 0.1% BSA, 14.6 mM $CaCl_2$, 0.07 mg/ml of phosphatidylcholine, and 0.03 mg/ml of phosphatidylserine) into plastic twin-well cuvettes. The cuvettes each contained 0.1 ml of the plasma preincubated with either 0.01 ml of buffer (control sample) or antibody (experimental sample) for 1-2 minutes. The inhibition of TF-mediated coagulation by the H36.D2 antibody was calculated using a TF standard curve in which the log [TF] was plotted against log clot time.

Fig. 5 shows the H36.D2 antibody substantially inhibits TF-initiated coagulation in human plasma. The H36.D2 antibody increased PT times significantly, showing that the antibody is an effective inhibitor of TF-initiated coagulation (up to approximately 99% inhibition).

EXAMPLE 6 - FX and the H36.D2 Antibody Compete For Binding to the TF:VIIa Complex

Competition experiments were conducted between TF/VIIa, FX and the H36.D2 antibody. Fig. 6A illustrates the results of an experiment in which a preformed TF/VIIa complex (0.08 nM) was pre-incubated at 37°C for 30 minutes in buffer including 0.02 nM, 0.04 nM, 0.08 nM and 0.16 nM of the H36.D2 monoclonal antibody, respectively. FX (30 nM) was then added to the TF/VIIa and H36.D2 antibody mixture and the mixture allowed to incubate for an additional 10 minutes at 37°C. FX activation was quenched with EDTA as described previously. The FXa produced thereby was determined by the FXa-specific assay described in Example 3, above.

Fig. 6B shows the results of an experiment conducted along the lines just-described, except that the H36.D2 antibody, pre-formed TF:VIIa, and FX were added simultaneously to start the FX activation assay.

The data set forth in Figs. 6A and 6B show that the H36.D2 antibody and FX compete for binding to the pre-formed TF/VIIa complex.

EXAMPLE 7 - Inhibition of TF Activity in Cell Culture

J-82 is a human bladder carcinoma cell line (available from the ATCC) which abundantly expresses native human TF as a cell surface protein. To see if the H36.D2 antibody could prevent FX from binding to native TF displayed on the cell surface, a J-82 FX activation assay was conducted in microtitre plates in the presence of FVII (see D.S. Fair et al., *J. Biol. Chem.*, **262**:11692 (1987)). To each well, 2 x 10^5 cells was added and incubated with either 50 ng FVII, buffer (control sample) or the anti-TF antibody (experimental sample) for 2 hours at 37°C. Afterwards, each well was gently washed with buffer and 0.3 ml of FX (0.05 mg/ml) was added to each well for 30 minutes at room temperature. In some cases, the antibody was added at the same time as FX to detect binding competition for the native TF. Thereafter, 0.05 ml aliquots were removed and added to new wells in a 96-well titre plate containing 0.025 ml of 100 mM EDTA. FXa activity was determined by the FXa-specific assay as described in Example 3, above. Inhibition of TF activity on the surface of the J-82 cells was calculated from the OD_{405nm} in the absence (control sample) and presence of antibody (experimental sample).

Fig. 7 shows that the H36.D2 antibody bound native TF expressed on J-82 cell membranes and inhibited TF-mediated activation of FX. These results indicate that the antibody competes with FX for binding to native TF displayed on the cell surface. Taken with the data of Example 8, below, the results also show that the H36.D2 antibody can bind a conformational epitope on native TF in a cell membrane.

EXAMPLE 8 - Specific Binding of the H36.D2 Antibody to Native rhTF

Evaluation of H36.D2 binding to native and non-native rhTF was performed by a simplified dot blot assay. Specifically, rhTF was diluted to 30 μg/ml in each of the following three buffers: 10 mM Tris-HCl, pH 8.0; 10 mM Tris-HCl, pH 8.0 and 8 M urea; and 10 mM Tris-HCl, pH 8.0, 8 M urea and 5 mM dithiothreitol. Incubation in the Tris buffer maintains rhTF in native form, whereas treatment with 8M urea and 5nM dithiothreitol produces non-native (denatured) rhTF. Each sample was incubated for 24 hours at room temperature.

After the incubation, a Millipore Immobilon (7x7cm section) membrane was pre-wetted with methanol, followed by 25 mM Tris, pH 10.4, including 20% methanol. After the membranes were air-dried, approximately 0.5 µl, 1 µl, and 2 µl of each sample (30 µg/ml) was applied to the membrane and air-dried. After blocking the membrane by PBS containing 5% (w/v) skim milk and 5% (v/v) NP-40, the membrane was probed with H36.D2 antibody, followed by incubation with a goat anti-mouse IgG peroxidase conjugate (obtained from Jackson ImmunoResearch Laboratories, Inc.). After incubation with ECL Western Blotting reagents in accordance with the manufacturer's instructions (Amersham), the membrane was wrapped with plastic film (Saran Wrap) and exposed to X-ray film for various times.

Fig. 8A shows that the H36.D2 Mab binds a conformational epitope on native TF in the presence of Tris buffer or Tris buffer with 8M urea (lanes 1 and 2). The autoradiogram was exposed for 40 seconds. However, when the native TF was denatured with 8M urea and 5mM DTT, H36.D2 binding was significantly reduced or eliminated (lane 3). Fig. 8B shows an over-exposed autoradiogram showing residual binding of the H36.D2 antibody to non-native (i.e., denatured) rhTF. The over-exposure was for approximately 120 seconds. Treatment with 8M urea alone probably resulted in only partial denaturation of the native rhTF since the two disulfide bonds in TF are not reduced. It is also possible that the partially denatured TF may refold back to native confirmation during later blotting process when urea is removed. These results also clearly distinguish preferred antibodies of the invention which do not bind denatured TF from previously reported antibodies which do not selectively bind to a conformational epitope and bind to denatured TF (see U.S. Patent 5,437,864 where in Figure 18 Western Blot analysis shows binding to TF denatured by SDS).

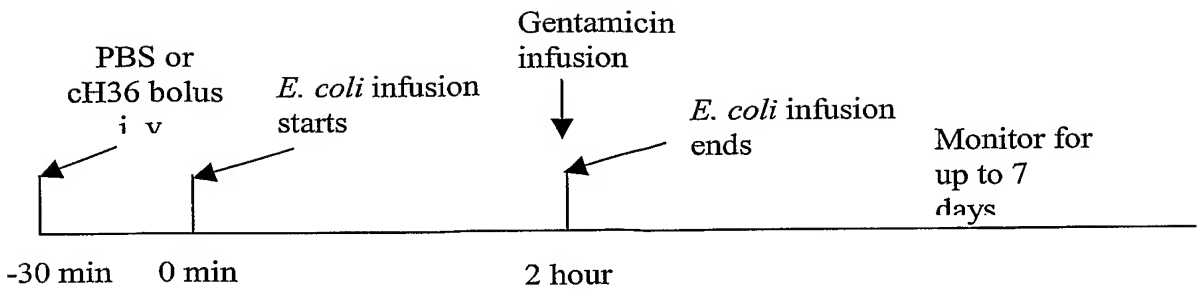
EXAMPLE 9: Septic Shock Model in Rhesus Monkeys

In this model, septic shock was induced by infusion of live *E. coli*, a gram-

negative bacterium (see Taylor *et al.*, J. Clin. Invest. **79**:918-825 (1987)) in rhesus monkeys. The shock induced by *E. coli* causes activation of both coagulation and inflammation, ultimately leading to death. The ability of an anti-TF antibody of the present invention to prolong the survival times of rhesus monkeys treated with live *E. coli* was examined using the rhesus model of septic shock described by Taylor *et al.*, *supra*. Rhesus monkeys weighing 3-5 kilograms were fasted overnight before study and immobilized the morning of the experiment with ketamine hydrochloride (14 mg/kg, intramuscularly). Sodium pentobarbital was then administered in the cephalic vein through a percutaneous catheter to maintain a light level of surgical anesthesia (2 mg/kg initially and with additional amounts approximately every 20 to 45 minutes for 6 hours). A femoral vein was exposed aseptically and cannulated for sampling blood and administering gentamicin. Gentamicin was administered by means of 30-minute intravenous infusions. An infusion of 9 mg/kg was administrated at the end of *E. coli* infusion (t = 2 hours). An infusion of 4.5 mg/kg was administrated 6 hours after *E. coli* infusion. Additional gentamicin (4.5 mg/kg, i.m.) was administrated once daily after day 1 for 3 days. Each monkey was placed on its side in contact with temperature controlled heating pads and rectal temperature was monitored. Animals were intubated orally and allowed to breathe spontaneously.

E. coli strain 086:K61H (ATCC 33985) was freshly prepared less than 12 hours prior to injection. Each monkey received a 2-hour intravenous infusion of *E. coli* at a dose of 4×10^{10} CFU/kg. Control group monkeys received PBS 30 minutes before infusion of *E. coli*. Treatment group monkeys received a bolus (2-3 minutes) dose of anti-tissue factor antibody (cH36, diluted in PBS if necessary) 30 minutes before infusion of *E. coli* (see Timeline for injection schedule). The percutaneous catheter was used to infuse *E. coli,* PBS and anti-TF antibody.

Timeline for anti-TF antibody and *E. coli* administration.



All monkeys were monitored continuously for 8 hours and observed daily for a maximum of 7 days, for the following: survival time: monitored and recorded hourly; temperature was measured and recorded hourly for the first 8 hours and then once a day for up to 7 days.

Blood samples were collected at the following time points: T = -0.5*, 0**, 1, 2, 4, 6, 24 hours as shown in Table 3 for the analysis of hematological references and inflammatory cytokines. (*T = -0.5, right before injection of cH36 or saline control; **T = 0, right before infusion of *E coli* but 30 min after injection of cH36 or saline control).

Table 1. The schedule for collecting blood samples.

Time Point	*Hematology*	*Plasma for Analysis*
Day 1; t = -0.5 hr (just prior to test article or vehicle infusion)	X	X
Day 1; t = 0 hour (30 minutes after treatment, just prior to *E. coli* infusion)	X	X
Day 1; 1 hour (following *E. coli* infusion	X	X
Day 1; 2 hour (following *E. coli* infusion)	X	X
Day 1; 4 hour (following *E. coli* infusion)	X	X
Day 1; 6 hour (following *E. coli* infusion)	X	X
Day 1; 24 hour (following *E. coli* infusion)	X	X
Volume of whole blood / time point	1.0 mL	1.8 mL
Anticoagulant	EDTA	Sodium Citrate

The results of this study are shown in Table 2. Anti-TF antibody cH36 protected rhesus monkeys from *E. coli*-induced septic shock when administered as a 10 mg/kg bolus injected intravenously (Table 2).

Table 2. Protective Effect of cH36 on *E coli*-induced Septic Shock in Rhesus Monkeys

Treatment	Weight (kg)	Sex	Survival Time (hr)	Average Survival Time (hr)
Saline	3.6 4.5	F M	8 24	16
Sunol-cH36 (10 mg/kg)	3.1 4.0	F M	>168 54	>111

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the disclosure, may make modification and improvements within the spirit and scope of the invention.

(1) GENERAL INFORMATION

(i) APPLICANT: Wong, Hing C.
Jiao, Jin-an
Esperanza, Nieves

(ii) TITLE OF THE INVENTION: ANTIBODIES FOR INHIBITING BLOOD COAGULATION AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE:
(B) STREET:
(C) CITY:
(D) STATE:
(E) COUNTRY:
(F) ZIP:

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

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(A) TELEPHONE: 617-523-3400
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACATTCAGA TGACCCAGTC TCCTGCCTCC CAGTCTGCAT CTCTGGGAGA AAGTGTCACC
60

ATCACATGCC TGGCAAGTCA GACCATTGAT ACATGGTTAG CATGGTATCA GCAGAAACCA
120
GGGAAATCTC CTCAGCTCCT GATTTATGCT GCCACCAACT TGGCAGATGG GGTCCCATCA
180
AGGTTCAGTG GCAGTGGATC TGGCACAAAA TTTTCTTTCA AGATCAGCAG CCTACAGGCT
240
GAAGATTTTG TAAATTATTA CTGTCAACAA GTTTACAGTT CTCCATTCAC GTTCGGTGCT
300
GGGACCAAGC TGGAGCTGAA A
321

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Gln Ser Ala Ser Leu Gly
1 5 10 15
Glu Ser Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Asp Thr Trp
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
35 40 45
Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Lys Phe Ser Phe Lys Ile Ser Ser Leu Gln Ala
65 70 75 80
Glu Asp Phe Val Asn Tyr Tyr Cys Gln Gln Val Tyr Ser Ser Pro Phe
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAGATCCAGC TGCAGCAGTC TGGACCTGAG CTGGTGAAGC CTGGGGCTTC AGTGCAGGTA
60
TCCTGCAAGA CTTCTGGTTA CTCATTCACT GACTACAACG TGTACTGGGT GAGGCAGAGC

120
CATGGAAAGA GCCTTGAGTG GATTGGATAT ATTGATCCTT ACAATGGTAT TACTATCTAC
180
GACCAGAACT TCAAGGGCAA GGCCACATTG ACTGTTGACA AGTCTTCCAC CACAGCCTTC
240
ATGCATCTCA ACAGCCTGAC ATCTGACGAC TCTGCAGTTT ATTTCTGTGC AAGAGATGTG
300
ACTACGGCCC TTGACTTCTG GGGCCAAGGC ACCACTCTCA CAGTCTCCTC A
351

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Gln Val Ser Cys Lys Thr Xaa Gly Tyr Ser Phe Thr Asp Tyr
20 25 30
Asn Val Tyr Trp Val Arg Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asp Pro Tyr Asn Gly Ile Thr Ile Tyr Asp Gln Asn Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Thr Ala Phe
65 70 75 80
Met His Leu Asn Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Phe Cys
85 90 95
Ala Arg Asp Val Thr Thr Ala Leu Asp Phe Trp Gly Gln Gly Thr Thr
100 105 110
Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Ala Ser Gln Thr Ile Asp
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Ala Thr Asn Leu Ala Asp
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Gln Val Tyr Ser Ser Pro Phe Thr
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Thr Asp Tyr Asn Val Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Ile Asp Pro Tyr Asn Gly Ile Thr Ile Tyr Asp Gln Asn Phe Lys
1 5 10 15
Gly

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Val Thr Thr Ala Leu Asp Phe
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGGCAAGTC AGACCATTGA T
21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCTGCCACCA ACTTGGCAGA T
21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CAACAAGTTT ACAGTTCTCC ATTCACGT
28

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACTGACTACA ACGTGTAC
18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TATATTGATC CTTACAATGG TATTACTATC TACGACCAGA ACTTCAAGGG C
51

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GATGTGACTA CGGCCCTTGA CTTC
24

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCACCTCCAG ATGTTAACTG CTC
23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAARTAVCCC TTGACCAGGC
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAGGCGGCG GTTCTGACAT TGTGMTGWCM CARTC
35

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTTCAGGCC CAGCCGGCCA TGGCCGARGT YCARCTKCAR CARYC
45

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCCGGGCCAC CATGKCCCCW RCTCAGYTYC TKG
33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCCGGGCCAC CATGGRATGS AGCTGKGTMA TSCTC

35

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATATACTCGC GACAGCTACA GGTGTCCACT CCGAGATCCA GCTGCAGCAG TC
52

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GACCTGAATT CTAAGGAGAC TGTGAGAGTG G
31

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTAATTGATA TCCAGATGAC CCAGTCTCC
29

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TAATCGTTCG AAAAGTGTAC TTACGTTTCA GCTCCAGCTT GGTCC
45

What is claimed is:

1. An antibody that binds native human tissue factor and does not substantially bind non-native tissue factor.

2. An antibody of claim 1 wherein the antibody has the binding specificity for native human tissue factor about equal to or greater than H36.D2.B7 [ATCC HB-12255].

3. An antibody having the binding affinity for native human tissue factor about equal to or greater than H36.D2.B7 [ATCC HB-12255].

4. An antibody having identifying characteristics of H36.D2.B7 [ATCC HB-12255].

5. An antibody of claim 1 wherein the antibody is H36.D2.B7 [ATCC HB-12255].

6. An antibody that binds native tissue factor to form a complex whereby factor X binding to the complex is inhibited.

7. An antibody of claim 1 wherein the antibody is a monoclonal antibody.

8. An antibody of claim 1 that is a chimeric antibody.

9. An antibody of claim 8 that comprises a constant region of human origin.

10. An antibody of claim 1 that is a single chain antibody.

11. An antibody that comprises a sequence that has at least about 70

percent sequence identity to SEQ ID NO:1.

12. An antibody of claim 11 that comprises a sequence represented by SEQ ID NO:2 or SEQ ID NO:4.

13. An antibody that comprises hypervariable regions that have at least 90 percent sequence identity to SEQ ID NOS. 5 through 10 inclusive.

14. An antibody of claim 13 wherein the antibody comprises hypervariable regions represented by SEQ ID NOS. 5 through 10 inclusive.

15. An isolated nucleic acid comprising a sequence encoding at least a portion of an antibody that binds native human tissue factor.

16. The nucleic acid of claim 15 wherein the monoclonal antibody is H36.D2.B7 [ATCC HB-12255].

17. The nucleic acid of claim 15 wherein the nucleic acid comprises SEQ ID NO:1 or SEQ ID NO:3.

18. The nucleic acid of claim 15 wherein the nucleic acid comprises a sequence that has at least about 70 percent sequence identity to SEQ ID NO:1 or SEQ ID NO:3.

19. The nucleic acid of claim 15 wherein the nucleic acid comprises sequences coding for antibody hypervariable regions that have at least 90 percent sequence identity to SEQ ID NOS. 5 through 10 inclusive.

20. A nucleic acid comprising at least about 100 base pairs and that hybridizes to SEQ ID NO:1 or SEQ ID NO:3 under normal stringency conditions.

21. A nucleic acid of claim 20 wherein the nucleic acid hybridizes to SEQ ID NO:1 or SEQ ID NO:3 under high stringency conditions.

22. A nucleic acid of claim 15 wherein the nucleic acid comprises sequences that have at least 90 percent sequence identitity to SEQ ID NOS. 11 through 16 inclusive and code for hypervariable regions.

23. A recombinant vector comprising the nucleic acid of claim 15, wherein the vector can express at least a portion of an antibody that binds native human tissue factor.

24. A host cell comprising the vector of claim 23.

25. A method of inhibiting blood coagulation in a mammal, comprising administering to the mammal an effective amount of an antibody capable of specifically binding native tissue factor and whereby the antibody complexes with native tissue factor, and factor X binding to the complex is inhibited.

26. The method of claim 25 wherein the complex further comprises factor VII/VIIa.

27. The method of claim 25 wherein the mammal is a human.

28. The method of claim 25 wherein the human is suffering from or suspected of having a thrombosis.

29. The method of claim 25 wherein the human is suffering from or susceptible to restenosis associated with an invasive medical procedure.

30. The method of claim 29 wherein the invasive medical procedure is angioplasty, endarterectomy, deployment of a stent, use of catheter, graft

implantation or use of an arteriovenous shunt.

31. The method of claim 25 wherein the human is suffering from a thromboembolic condition associated with cardiovascular disease, an infectious disease, a neoplastic disease or use of a thrombolytic agent.

32. The method of claim 25 further comprising administering an anti-platelet composition, a thrombolytic composition or an anti-coagulant composition.

33. The method of claim 25 wherein the antibody is H36.D2.B7 [ATCC HB-12255].

34. A method of reducing tissue factor levels in a mammal comprising:

administering to the mammal a therapeutically effective amount of an antibody capable of binding native tissue factor, the antibody linked covalently to a cell toxin or an effector molecule to provide complement-fixing ability and antibody-dependent cell-mediated cytotoxicity,

the antibody contacting cells expressing tissue factor to reduce tissue factor levels in the mammal.

35. The method of claim 34 wherein the cells expressing tissue factor are cancer cells, immune cells, or endothelial cells.

36. A method of detecting tissue factor in a biological sample comprising:

contacting a biological sample with a monoclonal antibody of claim 1 and analyzing the biological sample and monoclonal antibody for the presence of tissue factor in the biological sample.

H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTCACCATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C

CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L

CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T

AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAAATTATTACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y

AGTTCTCCATTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

* CDR REGIONS UNDERLINED.

FIG. 1A

H36.D2.B7 ANTI-TISSUE FACTOR HEAVY CHAIN VARIABLE REGION

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K
ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E

TGGATTGGATATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T

TTGACTGTTGACAAGTCTTCCACCACAGCCTTCATGCATCTCAACAGCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D D S A

GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGGGGCCAAGGCACCACTCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V

TCCTCA
S S

* CDR REGIONS UNDERLINED.

FIG. 1B

ANTIBODY	APPARENT K_d M^{-1}	APPARENT K_d M
BY ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
BY BIACore		
H36	3.1×10^{10}	3.2×10^{-11}
I43	2.3×10^9	4.3×10^{-10}
I47	3.2×10^9	3.1×10^{-10}
L133	4.6×10^9	2.2×10^{-10}
M107	1.1×10^9	9.1×10^{-10}

FIG. 2

ANTIBODY NAME	% INHIBITION ANTIBODY PREINCUBATED WITH TF/VIIa
D1	0
D1B	1
H31	4
H36	95
I43	1
J131	7
K80	0
K82	0
K87	1
L97B	7
L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

FIG. 3

ANTIBODY NAME	% INHIBITION TF PREINCUBATED WITH ANTIBODY PRIOR TO ADDITION OF VIIa	% INHIBITION TF PREINCUBATED WITH VIIa PRIOR TO ADDITION OF ANTIBODY
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

[rhTF],nM	[H36.D2],nM	H36.D2/rhTF MOLAR RATIO	CLOTTING TIME (SECONDS)	% INHIBITION OF rhTF FUNCTION
0.0048	0 1.61 3.23	0 335.4 670.8	102.3 114.3 121.3	0 31.3 45.8
0.023	0 1.61 3.23 6.45	0 70.0 140.0 280.4	77.6 85.3 91.1 99.6	0 52.2 65.2 73.9
0.092	0 3.23 6.45 12.90	0 35.1 70.1 140.2	49.3 65.8 88.5 113.3	0 65.2 90.2 95.7
0.46	0 6.45 12.90 32.30	0 14.0 28.0 70.2	32.6 52.7 80.2 117.9	0 82.4 96.7 99.3
2.30	0 16.10 32.30 64.50	0 7.0 14.0 28.0	23.9 47.1 95.2 115.3	0 94.4 99.7 99.9
11.52	0 16.10 32.30 64.50 161.30	0 1.4 2.8 5.6 14.0	22.2 30.2 46.0 87.6 114.0	0 93.4 98.8 99.9 100.0

FIG. 5

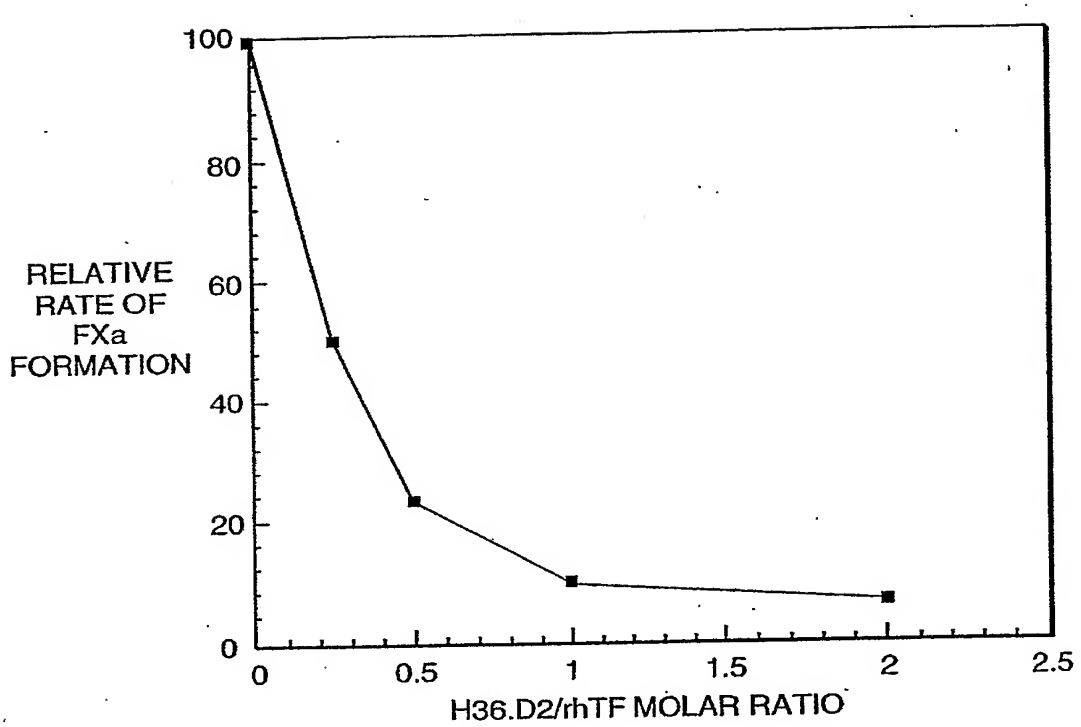
100
80
60
40
20
0
RELATIVE RATE OF FXa FORMATION
0
0.5
1
1.5
2
2.5
H36.D2/rhTF MOLAR RATIO

FIG. 6A

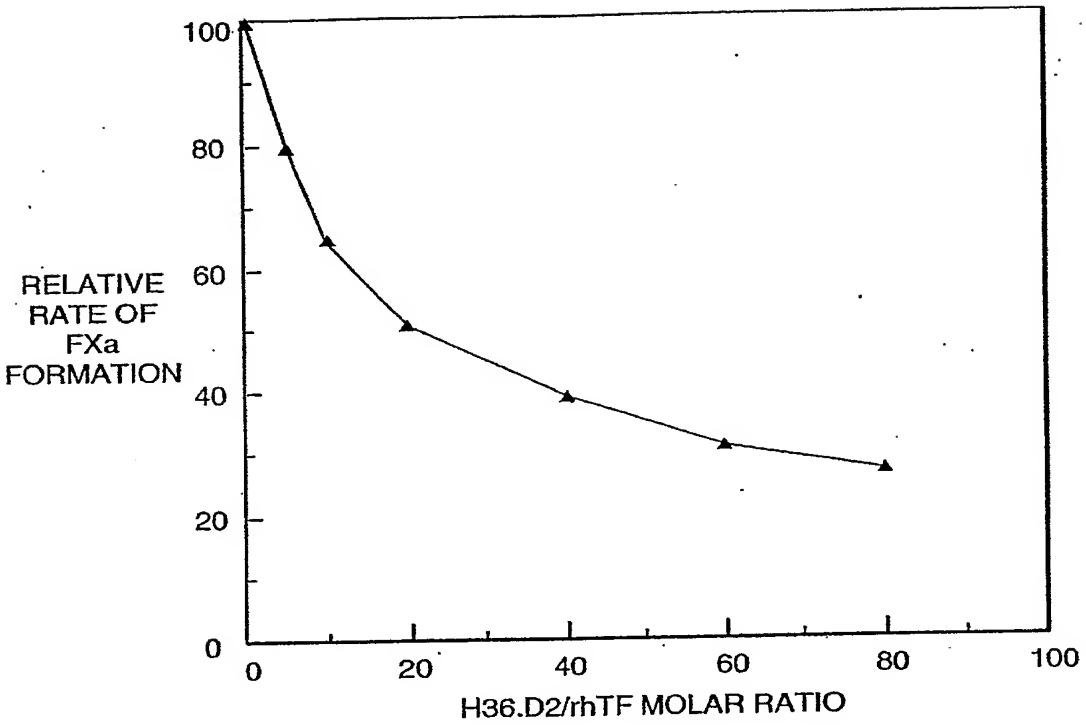
RELATIVE RATE OF FXa FORMATION
100
80
60
40
20
0
0
20
40
60
80
100
H36.D2/rhTF MOLAR RATIO

FIG. 6B

H36.D2 CONCENTRATION (ng)	% INHIBITION CELLS (TF/FVII) AND H36.D2 PREINCUBATED PRIOR TO FX ADDITION	% INHIBITION FX AND H36.D2 ARE ADDED SIMULTANEOUSLY TO CELLS (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

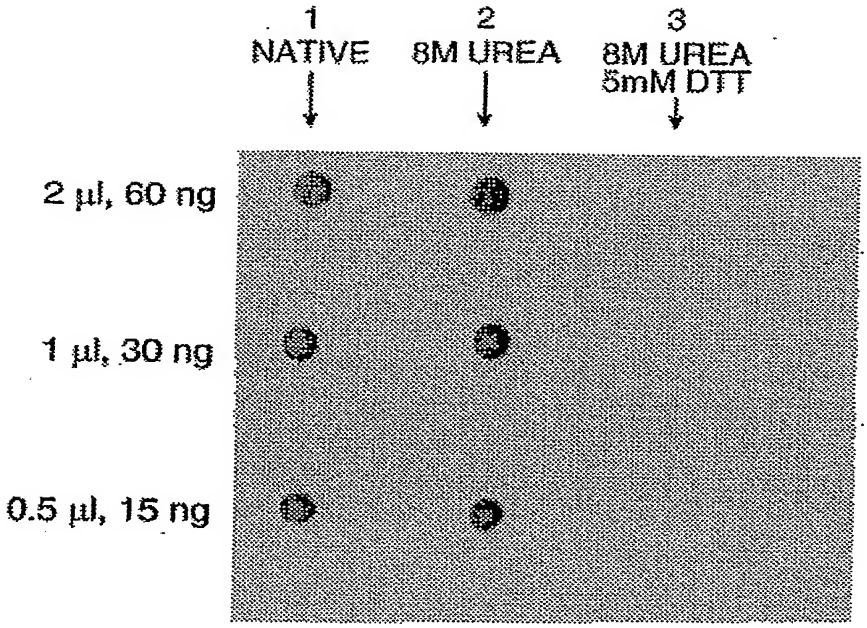
1
NATIVE
2
8M UREA
3
8M UREA
5mM DTT
2 µl, 60 ng
1 µl, 30 ng
0.5 µl, 15 ng

FIG. 8A

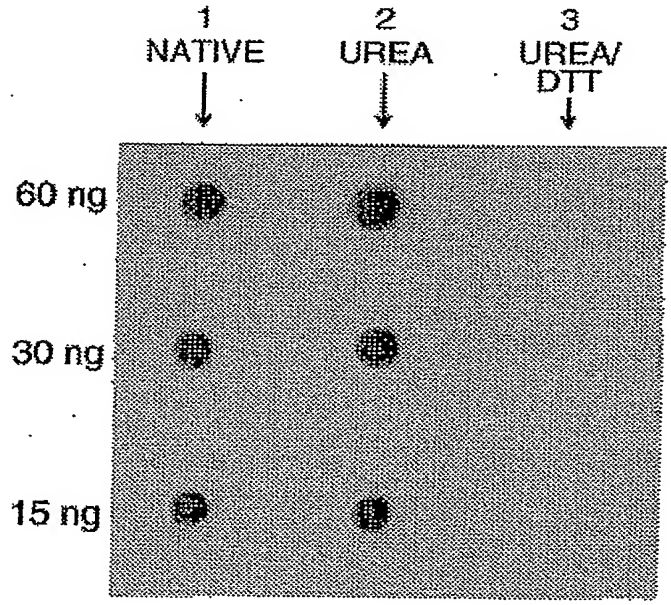
1
NATIVE
2
UREA
3
UREA/
DTT
60 ng
30 ng
15 ng

FIG. 8B

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/00 (2006.01)

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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18 May 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODIES FOR INHIBITING BLOOD COAGULATION AND METHODS OF USE THEREOF

(57) Abstract: Disclosed is a method for treating blood coagulation in a mammal that has or is suspected of having septic shock syndrome. In one embodiment, the method includes administering to the mammal an effective amount of an antibody that binds tissue factor in a way that excludes Factor X (FX) binding. The invention has a wide range of useful applications including use to inhibit unwanted blood coagulation associated with sepsis.

WO 2005/072126 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/01116

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C07K 16/00**(2006.01);**C12P 21/08**(2006.01);**C12N 15/00**(2006.01),**5/16**(2006.01);**A61K 39/395**(2006.01)

USPC: 530/387.1,387.3,388.1;435/320.1,326;424/130.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/387.1, 387.3, 388.1; 435/320.1, 326; 424/130.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN (Medline, Biosis), STIC (sequence)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,437,864 A (EDGINGTON et al.) 01 August 1995 (01.08.1995), abstract; column 22 lines 53-54; column 23, lines 15-18 and 48-51; column 25, lines 26-33; column 34, lines 48-54; and columns 56-57, Example 24; column 23, line 67 to column 24, line 2.	1-4, 6, 7, 10, 25-32 --- 8 and 9
Y	RIECHMANN, L. et al. Reshaping human antibodies for therapy. Nature, 24 March 1988, Vol 332, pages 323-327, especially pages 323 and 325-327.	8 and 9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
03 March 2007 (03.03.2007)

Date of mailing of the international search report
27 MAR 2007

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Form PCT/ISA/210 (second sheet) (April 2005)

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-33, drawn to an antibody binding native human tissue factor but not non-native human tissue factor, the encoding nucleic acid, a vector containing the nucleic acid, a host cell thereof, and a method of inhibiting blood coagulation by administering said antibody.

Group II, claim(s) 34 and 35, drawn to a method of reducing tissue factor levels by administering said antibody conjugated to a cell toxin, providing complement-fixing ability and cell-mediated cytotoxicity.

Group III, claim(s) 36, drawn to a method of detecting tissue factor in a biological sample using said antibody.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

This authority considers that the main invention in the instant application comprises the first-recited product, an antibody, and the first-recited method of using the antibody, namely a method of inhibiting blood coagulation with the antibody. Also included in this group is a nucleic acid encoding the antibody, a recombinant vector comprising the nucleic acid, and a host cell thereof. The additional method of group II does not correspond to the main invention, as it is drawn to a method using a distinct product, i.e., the antibody linked to a cell toxin, and being for a distinct purpose, i.e., for cytotoxicity. Therefore, the two groups are not considered to share a special technical feature within the meaning of PCT Rule 13.2, and thus do not relate to a single invention concept within the meaning of PCT Rule 13.1. The inventions listed as Groups I and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features because of the following: 37 CFR 1.475(d) states that if multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the main invention in the claims, see PCT Article 17(3)(a) and 1.476 (c). Claims 25-33 are drawn to the first method of using the product of claim 1 and will be grouped with the product as the main

invention. Group III requires the "special technical feature" such as that it is carried out in vitro, and requires detecting step, which are not required for the method in Group I. The method in Group I requires the "special technical feature" of administering the antibody to a patient, which is not required for Group III. Unity is lacking between Groups I and III.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/01116

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-33

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.